[CANCER RESEARCH 53, 721–724, February 15, 1993]

Advances in Brief

Metabolites of the Tobacco-specific Nitrosamine 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone in Smokers' Urine[1]

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Abstract

Metabolites of the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a potent pulmonary carcinogen, have been quantified in the urine of 11 smokers. They were not detected in nonsmokers' urine. The metabolites, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronide, were detected in quantities of 0.23–1.0 and 0.57–6.5 μg/24 h, respectively. The results of this study provide the first evidence for metabolites of tobacco-specific nitrosamines in human urine.

Introduction

It has been estimated that up to 90% of lung cancer deaths in the United States are attributable to cigarette smoking (1). The tobacco-specific nitrosamine NNK[2] (Fig. 1) is believed to play an important role in the induction of lung cancer in smokers because it is a potent pulmonary carcinogen in rats, mice, and hamsters inducing tumors at total doses similar to the estimated doses to which smokers are exposed (2, 3). NNK may also be involved in oral and pancreatic cancer associated with the use of tobacco products (3, 4). Although the metabolism of NNK has been extensively studied in laboratory animals, relatively little is known about its uptake and metabolism in humans. We believe that such information is critical to an understanding of mechanisms of cancer induction in humans.

In rodents and monkeys, identified pathways of NNK metabolism include α-hydroxylation, pyridine-*N*-oxidation, carbonyl reduction to NNAL, and conjugation of NNAL to the diastereomeric glucuronides NNAL-Gluc(I) and NNAL-Gluc(II) (Fig. 1) (2, 5, 6). Previous studies using cultured human tissues or microsomes have shown that NNK is metabolized by α-hydroxylation and carbonyl reduction (7, 8). Hemoglobin and DNA adducts resulting from α-hydroxylation of NNK, NNAL, or the related carcinogen NNN have been detected in the blood of smokers or snuff-dippers (9, 10). In this article, we present the first evidence for the presence in smokers' urine of NNK metabolites: NNAL; NNAL-Gluc(I); and NNAL-Gluc(II). Quantitation of NNK metabolites in human urine should greatly facilitate our understanding of its role in tobacco-related cancers.

Materials and Methods

Chemicals. [5-^{2}H]NNAL was obtained by $NaBH_4$ reduction of [5-^{2}H]NNK (Chemsyn Science Laboratories, Lenexa, KS). [5-^{2}H]NNAL-Gluc(II) was isolated from the urine of a patas monkey treated with [5-^{2}H]NNK (6). NNAL, iso-NNAL, nitrosoguvacoline, and 4-(methylamino)-4-(3-pyridyl)-1-butanol were synthesized (11–13). Bis-trimethylsilyltrifluoroacetamide/1% trimethylchlorosilane was obtained from Regis Chemical Co. (Morton Grove, IL). β-Glucuronidase, type IXA, sulfatase, type VIII, and saccharic acid 1,4-lactone were obtained from Sigma Chemical Co. (St. Louis, MO). 3-Acetylpyridine and 2-pyridylcarbinol were obtained from Aldrich Chemical Co. (Milwaukee, WI). The latter was converted to its acetate by treatment with acetic anhydride and triethylamine in CH_2Cl_2.

Apparatus. HPLC was performed using a Millipore Waters Division system as previously described (9) and a 3.9- × 300-mm Bondclone 10 C18 column (Phenomenex, Torrance, CA) with UV detection at 254 nm. Solvent A was H_2O and solvent B was methanol. The solvent program was 15% B in A for 10 min, then to 45% B in A over 30 min, then back to initial conditions in 5 min, and then held for 25 min prior to the next injection.

GC-TEA was performed with a Model 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) interfaced with a Model 610 Thermal Energy Analyzer (Thermedics Inco, Woburn, MA) and a Model D-2000 integrator (Hitachi Instruments, Danbury, CT) (14). The gas chromatograph was equipped with a 2-mm × 12-ft glass column filled with 3% XE-60 on GasChrom Q, 100/200 mesh (Alltech/Applied Sciences, Deerfield, IL). The oven was temperature-programmed as follows: 150°C for 3 min; then 6°C/min to 220°C; and then held for 15 min. The injection port temperature was 230°C and the flow rate was 33 ml/min argon.

GC-MS-SIM was carried out with a Hewlett-Packard Model 5988A instrument, operated in the positive chemical ionization mode with a methane pressure of 0.88 torr, an ionizing energy of 107 eV, and a source temperature of 200°C. For electron impact experiments, the ionizing energy was 70 eV and the source temperature was 200°C. The analyses were performed by splitless injection on a 0.25-mm × 30-m Econocap SE 54 column (film thickness, 0.25 μm; Alltech/Applied Sciences), with a 0.32-mm × 1-m retention gap. The carrier gas was He (head pressure, 12 psi) and the oven temperature was programmed as follows: 100°C for 1 min; then 8°C/min to 180°C; and then held for 30 min.

Volunteers. Eleven smokers and 7 nonsmokers ranging in age from 20 to 65 years were recruited. The protocol for collection of urine was approved by the American Health Foundation Institutional Review Board for protection of human subjects.

Analysis of Urine by GC-TEA. Twenty-four-h urine samples were collected in 3 liter amber specimen containers (Baxter Scientific Products Division, McGaw Park, IL) to which 10 ml of a solution of 20% ammonium sulfamate (Sigma) in 3.6 *N* H_2SO_4 had been added to inhibit artifactual nitrosation. Samples were stored at room temperature during collection. Aliquots of 100 ml were adjusted to pH 7 with 10 *N* NaOH. To this was added 0.5 ml of an aqueous solution of [5-^{2}H]NNAL-Gluc(II) (2.1 Ci/mmol; 21,000 dpm) as internal standard. The resulting solution was extracted 3 times with equal volumes of ethyl acetate. The aqueous portion (A-1) was saved. A solution of [5-^{2}H]NNAL (2.1 Ci/mmol; 100,000 dpm) in 50 μl of methanol was added to the combined ethyl acetate extracts, as internal standard for unconjugated NNAL. The ethyl acetate layers were dried (Na_2SO_4) and concentrated to dryness by rotary evaporation. The residue (Fig. 2, Fraction 1) was dissolved in two 0.5-ml aliquots of H_2O, which were combined and set aside for subsequent HPLC purification and analysis for NNAL. Twenty μl of "antifoam B emulsion" (Sigma) were added to the aqueous portion, A-1, which was then concentrated by rotary evaporation (water bath temperature, 35°C) to approximately 70% of its initial volume. The purpose of this step was removal of most of the ethyl acetate, traces of which inhibited β-glucuronidase

Received 11/17/92; accepted 12/31/92.

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[1] This study was supported by Grant CA-29580 from the National Cancer Institute.

[2] The abbreviations used are: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc(I) and NNAL-Gluc(II), two diastereomers of [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-D-glucosiduronic acid; NNAL-TMS, trimethylsilyl ether of NNAL; iso-NNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; HPLC, high performance liquid chromatography; GC-TEA, combined gas chromatography-thermal energy analyzer; GC-MS-SIM, combined gas chromatography-mass spectrometry-selected ion monitoring.

PM3001192659

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